ASSEMBLY OF NON-NEURAL MICROTUBULES IN THE PRESENCE OF CALCIUM IONS

Karl H. DOENGES
Institute for Cell Research, German Cancer Research Centre, D-6900 Heidelberg, FRG

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1. Introduction

Since the first report [1], that cytoplasmic microtubules could be polymerized from mammalian brain if calcium ions were removed with chelators, considerable progress has been made on the chemistry of tubulin and the mechanism of assembly. It has been shown that the in vitro assembly of brain tubulin can be reversibly controlled by Ca^{2+} [1–4]. These results have aroused much interest with regard to the possible function of Ca^{2+} as a regulator of assembly in vivo. Relatively little is known, however, about tubulin assembly in extracts from other types of cells. Recently we described the in vitro assembly of tubulin from non-neural Ehrlich ascites tumour (EAT) cells [5] and other cultured cells [6], and presented evidence that the assembly model based on neurotubulin can not be generalized. Here we show that tubulin from EAT cells can readily assemble into microtubules in the absence of Ca^{2+} chelators, and that this assembly is not blocked by low or high concentrations of Ca^{2+}.

2. Materials and methods

Tubulin from EAT cells was prepared as in [7] as modified [5] in buffer containing 0.1 M MES (2-(*N*-morpholino)ethanesulfonic acid), 0.5 mM $MgCl_2$, pH 6.5. In experiments to be done in the presence of chelators, MES buffer was supplemented with 1 mM EGTA (ethyleneglycol-bis(2-aminoethyl ether)*N,N'*-tetraacetic acid) and 0.1 mM EDTA (ethylenediaminetetraacetic acid).

Protein concentrations were determined as in [8], using bovine serum albumin as a standard.

Polyacrylamide–sodium dodecylsulfate gel electrophoresis was carried out on slab gels as in [9].

Polymerization of microtubules was measured by using the turbidity assay developed [10]. The measurements were made at A_{350}; microtubule assembly was induced by adding GTP and elevating the temperature of tubulin solutions from 0–37°C.

Samples for electron microscopy were applied to carbon-coated Formvar films cast on copper grids, stained with 2% uranylacetate and examined in a Siemens 1A electron microscope.

3. Results and discussion

Assembly of microtubules from EAT cells was observed in the absence and presence of the Ca^{2+} chelators EGTA and EDTA, showing no differences in the yields of 2 times polymerized tubulin (approx. 25–30 mg protein for 200 g EAT cells) (fig.1). This result indicates that μM levels of Ca^{2+} do not inhibit microtubule assembly. In order to test if the formation of non-neural microtubules is at all sensitive to Ca^{2+}, experiments were carried out to determine the effect of Ca^{2+} on the extent of polymerization using turbidity measurements and electron microscopy. Microtubule protein was isolated by 2 cycles of polymerization-depolymerization in assembly buffer without EGTA and EDTA. The protein solution was made 1 mM in GTP and the incubation was carried out at 37°C in the presence of varying concentrations of Ca^{2+} (fig.2). At concentrations of Ca^{2+} between 10^{-5} M and 10^{-3} M a slight inhibition of polymerization (10–15%) is observed spectrophotometically. Concentrations of Ca^{2+} above 10^{-3} M cause a rise in turbidity which increases with increasing concentra-

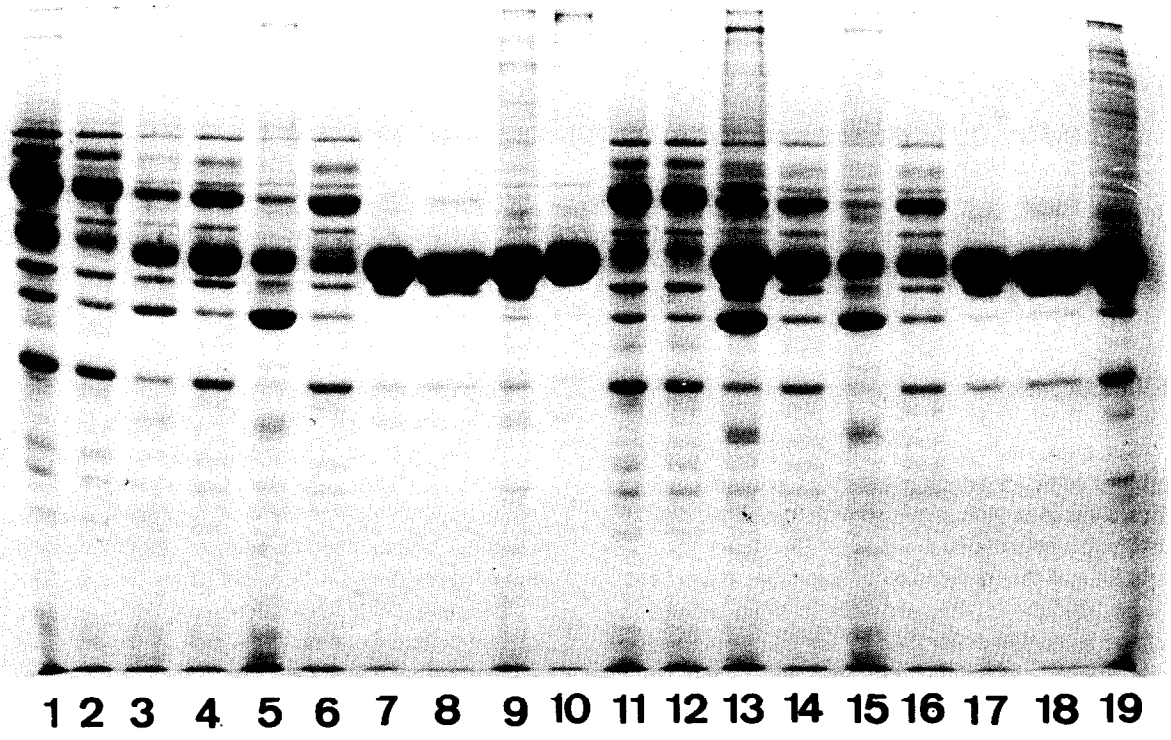
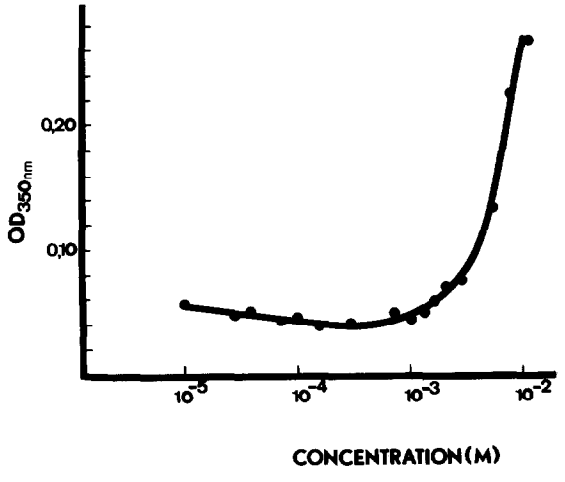


Fig.1. Analysis of microtubule proteins by sodium dodecylsulfate–polyacrylamide gel electrophoresis. (1–9) Fractions prepared with EGTA and EDTA. (11–19) Fractions prepared without EGTA and EDTA. (1, 11) Whole extracts. (2, 12) Supernatants after first assembly. (3, 13) Pellets after first assembly. (4, 14) Supernatants after first disassembly. (5, 15) Pellets after first disassembly. (6, 16) Supernatants after second assembly. (7, 17) Pellets after second assembly. (8, 18) Supernatants after second disassembly. (9, 19) Pellets after second disassembly. (10) Two-times cycled porcine brain tubulin.

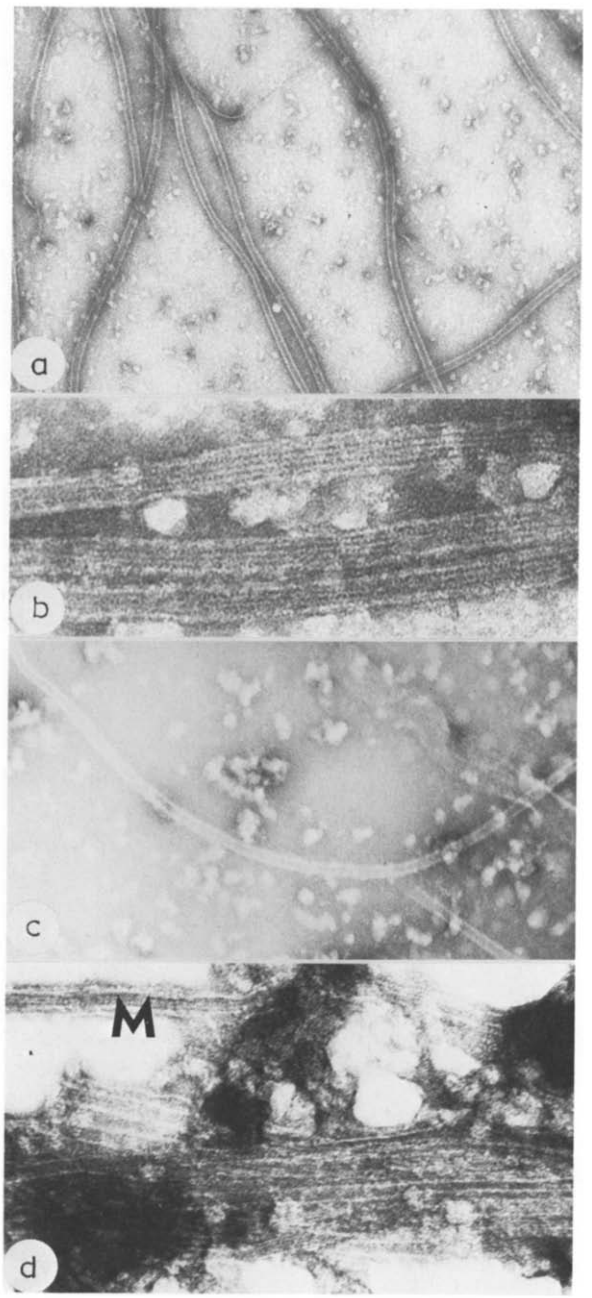


tions of Ca^{2+}. In contrast to these results, 2-times cycled tubulin from porcine brain was totally inhibited at Ca^{2+} levels greater than 2×10^{-3} M. This inhibition of neural tubulin by Ca^{2+} at mM levels has been described [2].

At low levels of Ca^{2+}, between 10^{-5} M and 10^{-3} M, negatively-stained specimens examined in

Fig.2. Effect of Ca^{2+} on the turbidity of 2-times cycled EAT tubulin in MES buffer without Ca^{2+} chelators. Immediately before incubation, aliquots were made to various concentrations with $CaCl_2$. Polymerization of microtubules was induced by addition of 1 mM GTP to protein solutions (0.5 mg/ml) and elevating the temperature from 0°C to 37°C. Absorbance $(OD)_{350\ nm}$ represents the plateau turbidity values. The control $OD_{350\ nm}$ of protein polymerized in the absence of Ca^{2+} and with or without EGTA/EDTA was 0.050.

the electron microscope show the presence of large amounts of microtubules (fig.3a). At higher concentrations of Ca^{2+} (2×10^{-3} M to 10^{-2} M) long intact microtubules, a number of sheet polymers and some macrotubules averaging 45 nm diam. were always present (fig.3b–d). The appearance of sheets and macrotubules obviously accounts for the large increase in turbidity at high concentrations of Ca^{2+}. Similar findings were made [11] for dogfish-brain tubulin polymerized using similar concentrations of Ca^{2+}. It has been reported that high concentrations of Ca^{2+} induce [11] and stabilize [12] rings of aggregated tubulin from brain tissue. In contrast to neural tubulin we have been unable to find ring-like structures in EAT tubulin [5], even under high concentrations of Ca^{2+} (fig.3b–d). However, we found that EAT tubulin does form rings when brain microtubule associated proteins (MAPs) are added [13]. We have described a similar observation in preparations of tubulin from cultured cells [6].



Preliminary results show that the polymerization of EAT tubulin can be inhibited by mM levels of Ca^{2+} on addition of MAPs from neural tubulin. Furthermore, reciprocal mixing of brain tubulin with EAT-MAPs results in an increased Ca^{2+} insensitivity of neural microtubule protein. It seems possible that the different Ca^{2+} sensitivities between neural and non-neural microtubules may be a result of the differences in MAPs [5]. There have already been some disagreements over the concentration range at which Ca^{2+} inhibits the assembly of neurotubules [2,14]. Ca^{2+}-sensitizing factor(s) present in crude extracts and abandoned during several cycling steps could account for the reported discrepancies in the neurotubulin system [15]. We are currently testing this possibility by looking for a Ca^{2+}-sensitizing factor(s) and have begun an analysis of the microtubule accessory proteins. There is increasing evidence that Ca^{2+} may regulate the function of proteins involved in non-muscle cellular movements, such as troponin, actin and myosin [16]. If these proteins influence the assembly of microtubules this would signify an indirect regulation of tubulin polymerization by Ca^{2+}.

Fig.3. Electron micrographs of microtubules polymerized in the presence of Ca^{2+}. Two-times cycled EAT tubulin (0.5 mg protein/ml) was incubated 30 min at 37°C in MES buffer with 1 mM GTP and different concentrations of $CaCl_2$. (a) Microtubules formed in the presence of 5×10^{-5} M $CaCl_2$; × 44 000. (b) Microtubules at 2×10^{-3} M $CaCl_2$; × 176 000. (c) A sheet is present along with completely closed microtubules at 5×10^{-3} M $CACl_2$; × 54 000. (d) Long sheet polymers and macrotubules (M) at 10^{-2} M $CaCl_2$; × 105 600. No rings could be observed.

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References

[1] Weisenberg, R. C. (1972) Science 177, 1104–1105.
[2] Olmsted, J. B. and Borisy, G. G. (1975) Biochemistry 14, 2996–3005.
[3] Kuriyama, R. and Sakai, H. (1974) J. Biochem. (Tokyo) 75, 463–471.
[4] Haga, T., Abe, T. and Kurokawa, M. (1974) FEBS Lett. 39, 291–295.
[5] Doenges, K. H., Nagle, B. W., Uhlmann, A. and Bryan, J. (1977) Biochemistry 16, 3455–3459.
[6] Nagle, B. W., Doenges, K. H. and Bryan, J. (1977) Cell 12, 573–586.
[7] Shelanski, M. L., Gaskin, F. and Cantor, C. R. (1973) Proc. Natl. Acad. Sci. USA 70, 765–768.
[8] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[9] Laemmli, U. K. (1970) Nature 227, 680–685.
[10] Gaskin, F., Cantor, C. R. and Shelanski, M. L. (1974) J. Mol. Biol. 89, 737–758.
[11] Langford, G. M. (1978) Exp. Cell Res. 111, 139–151.
[12] Weisenberg, R. C. (1974) J. Supramol. Struc. 2, 451–465.
[13] Doenges, K. H., unpublished results.
[14] Rosenfeld, A. C., Zackroff, R. V. and Weisenberg, R. C. (1976) FEBS Lett. 65, 144–147.
[15] Nishida, E. and Sakai, H. (1977) J. Biochem. (Tokyo) 82, 303–306.
[16] Taylor, D. L. (1976) in: Cell Motility, (Goldman, R., Pollard, T. and Rosenbaum, J. eds) pp. 797–821, Cold Spring Harbor Lab., Cold Spring Harbor.